



Beutner
Laboratories

Reference Manual

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**Beutner
Laboratories**

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General Information

INTRODUCTION

KSL Beutner Laboratories was founded by [Ernst H. Beutner, Ph.D.](#) and Gloria Beutner in 1992. Development of defined, quantified Immunofluorescent (IF) methods now used for studies of autoimmune skin diseases started with studies of experimental autoimmune thyroiditis at the State University of New York at Buffalo (UB) under the leadership of Ernest Witebsky, M.D. and with guidance from Albert Coons, M.D. at Harvard University.

From 1968 to 1992 Tadeusz Chorzelski, M.D. and his chief, Stefania Jablonska, M.D. of the Warsaw Medical School, Poland joined with Beutner's group at UB in studies that yielded over 125 publications on autoimmunity in skin disease, 5 edited books and 8 summer teaching programs in the 1970's primarily for dermatologists on uses of IF methods in studies of skin immunopathology. Also, from 1965 to 1985 Beutner and his associates at UB led an international effort to introduce defined IF into diverse immunologic studies by quantifying fluorescein to protein ratios and labeled antibody protein content of fluorescein conjugated antibodies. This formed the foundation for the present day reproducible defined immunofluorescence.

Dermal and mucosal autoimmunity has been our focus. Supported by our extensive research and a proven track record, KSL Beutner Laboratories is a specialized reference laboratory for immunopathology of autoimmune bullous and connective tissue diseases, oral pathology, dermatopathology and immunology tests pertaining to dermal oncology. KSL Beutner Laboratories immunology laboratory provides testing for an extensive list of prevalent and esoteric autoimmune diseases using cutting edge diagnostics. We use multiple technologies including direct immunofluorescence (DIF) and indirect immunofluorescence (IIF), enzyme linked immunosorbent assays (ELISA), and immunoblot detection methods to support healthcare practitioners across a wide clinical spectrum of autoimmune bullous and connective tissue diseases.

LICENSURE AND ACCREDITATION

KSL Beutner Laboratories is CLIA and CAP accredited and licensed by the New York State Department of Health, as well as the states of California, Maryland, Pennsylvania, and Rhode Island.

STANDARDS OF SERVICE

KSL Beutner Laboratories is known for high quality, timely and accurate reports, keen consultations and impeccable customer service. Biological specimens are collected by complimentary courier or express mail service. Reports can be viewed at KSL Beutner Laboratories online, a convenient HIPAA compliant lab information system. Our laboratory also offers flexible billing options to suit your needs. Healthcare professionals have come to know the expert capabilities of our specialized laboratory, along with our outstanding and personal service.

HOLIDAY COVERAGE

KSL Beutner Laboratories observes the following holidays: New Year's Day, Memorial Day, Independence Day, Labor Day, Thanksgiving Day, Day After Thanksgiving, Christmas Eve and Christmas Day. Please be aware that we are not staffed on these holidays. Should you have any questions or concerns, please contact Customer Care between the hours of 8 AM and 4 PM prior to the holiday. Commercial courier services may also be delayed or closed on these major holidays, so please check with these couriers before shipping samples.

MEDICAL TEAM

The KSL Beutner Laboratories Medical team draws upon exclusive clinical and research experience, frequently contributing to the scientific literature in the field of immunology. Members of our staff include PhD's, DDS's and MD's, and are certified by the American Board of Medical Microbiology, the American Board of Medical Laboratory Immunology, and American Board of Oral and Maxillofacial Pathology. Clients have ready access to pathologists with expertise in immunopathology, and oral pathology for consultations.

BIOGRAPHIES



Raminder Grover, MD, (D)ABMM | Laboratory Director

Dr. Raminder Grover is a Diplomate of the American Board of Medical Microbiology. She is certified by the New York State Department of Health in Diagnostic Immunology and has years of clinical laboratory experience. She worked with Dr. Beutner for years gaining experience in diagnostic studies for autoimmune skin diseases. She specializes in skin immunopathology, immunofluorescence assays, ELISA and immunoblot assays for the diagnosis of autoimmune skin and mucous membrane diseases. She is a volunteer faculty in the Department of Dermatology at SUNY, Buffalo and also has authored research papers in skin autoimmunity, microbiology and virology.



Lakshmanan Suresh, DDS, MS, PhD, (D)ABMLI, (D) ABOMP | Technical Director

Dr. Suresh is board-certified in Oral and Maxillofacial Pathology by the American Board of Oral and Maxillofacial Pathology. He is also board certified in Laboratory Immunology by American Board of Medical Laboratory Immunology. He has authored numerous publications and book chapters, holds several patents and maintains research collaborations around the world. He is a Clinical Professor at the State University of New York at Buffalo.



Jill M. Kramer, DDS, PhD, (D)ABOMP | Assistant Director

Dr. Kramer received her PhD and DDS degrees from the University at Buffalo, State University of New York. She completed her specialty training in Oral and Maxillofacial Pathology at Long Island Jewish Medical Center and her post-doctoral studies at the Feinstein Institute for Medical Research. She is a fellow in the American Academy of Oral and Maxillofacial Pathologists and a diplomate in the American Board of Oral and Maxillofacial Pathology. Dr. Kramer is currently an associate professor in the Department of Oral Biology and she holds adjunct appointments within the Departments of Oral Diagnostic Sciences and Microbiology and Immunology at the University at Buffalo. Dr. Kramer conducts NIH-funded research in the area of immune dysfunction in Sjögren's syndrome and she has authored numerous manuscripts detailing the role of immune cells in this disease. She serves as a reviewer for many scientific journals and is an active member of several scientific societies, including the American Association for Dental, Oral and Craniofacial Research, the American Association of Immunologists and the Society for Leukocyte Biology. Dr. Kramer has worked as a diagnostic immunologist and oral pathologist since 2014.

BILLING INFORMATION

KSL Beutner Laboratories will bill hospitals, reference laboratories, clinics or medical groups. Alternatively, KSL Beutner Laboratories will bill patients' insurance directly, provided all the necessary billing information is supplied at the time services are rendered (See requirements listed below).

1. KSL Beutner Laboratories does not have capitation contract agreements with any HMO's. Due to Knox-Keene regulations, if a third-party payer is initially billed and is denied as an HMO member, these charges will be billed back to the patients.
2. KSL Beutner Laboratories will bill Medicare for tests performed at KSL Beutner Laboratories. If a claim is denied as "not eligible for the specified date(s) of service", the charges will be billed to the patient.
3. Changes to Billing instructions must be supplied within 30 days from the date of the invoice. KSL Beutner Laboratories will not process any billing instruction change requests received after the 30-day period. The charges will remain the patient's responsibility.

If local or state requirements preclude providing the patient's name to ensure confidentiality, KSL Beutner Laboratories will be unable to bill patients' insurance directly; the charges will be billed to the client. All billing discrepancies should be reported to our Billing Department immediately. Our Billing Department is available from 8:00am to 3:30pm EST by calling 1-800-288-0549. Our Billing Specialists are available to answer questions and resolve any problems. All bills are due and payable upon receipt.

We are able to bill all insurances and for the list of insurances we participate in, please call our billing department at 1-800-288-0549.

KSL Beutner Laboratories Federal I.D. number is 16-1596380

Professional Courtesy

"Professional Courtesy" testing is strictly prohibited as stated in the Anti-Kick Back Statute U.S.C. 1320a – 7b; therefore, KSL Beutner Laboratories cannot honor request for this service.

Patient Billing

KSL Beutner Laboratories can bill the patient's insurance directly if complete billing information is provided on the Test Requisition Form or a copy of insurance card, front and back, is included at the time the specimen is submitted. KSL Beutner Laboratories Patient Statements are issued immediately following response from insurance. The patient is solely responsible for the charges. Patient bills are due upon receipt.

Third Party Billing

KSL Beutner Laboratories can bill the patient's insurance company directly for tests performed by KSL Beutner Laboratories if the information listed below is provided. KSL Beutner Laboratories will not bill third party payers for referral testing submitted to KSL Beutner Laboratories for performance by a send out laboratory.

Billing Information Requirements (to be entered on KSL Beutner Laboratories Test Requisition Form):

- Patient Name
- Patient Date of Birth
- Patient Sex
- Patient Address, including City, State, Zip (if billing a facility, address is not needed)
- Patient Signature
- Patient Relationship to Subscriber
- Insurance Carrier Name
- Insurance Carrier Address, including City, State, Zip
- Subscriber Name and Date of Birth
- Policy Number or Members ID Group Number
- Requesting Physician Name
- Physician (Provider) Signature
- Requesting Physician NPI #
- Diagnosis (ICD-10 Code) applicable to the patient's condition at time of service

Patients are responsible for the yearly deductibles, co-payments and any balance not covered by the insurance company. If insurance payment is not received within 60 days, the patient is billed directly.

Medicare Billing

KSL Beutner Laboratories is a Medicare Provider. If your patient has Medicare coverage, please send us complete information including secondary insurance, if applicable. KSL Beutner Laboratories will bill Medicare and accept 80% assignment. Complete information, including the "New" Medicare number must be entered on the Test Requisition Form or a copy of insurance cards front and back at the time the specimen is submitted. *Please note: Due to HIPAA Transaction Code Standards effective October 16, 2003, a valid diagnosis code is mandatory for billing Medicare. Medicare billing information is not complete and will not be accepted without a valid diagnosis code.*

LMRP and NCD Requirements

Medicare tests listed on the National Coverage Determinants (NCD) & Local Medical Review Policies (LMRP) will not be reimbursed by Medicare without a covered diagnosis code applicable to the patient at time of service. If a diagnosis code cannot be provided that matches the NCD or LMRP requirement, an Advance Beneficiary Notice (ABN) should be obtained from the patient and forwarded with the requisition.

Referrals from Hospitals

Under Medicare rules, KSL Beutner Laboratories can only bill Medicare for a hospital-referred test when the specimen was not collected as part of an inpatient or outpatient encounter, i.e., the specimen was not drawn in a hospital facility or by hospital personnel. All other testing for hospital patients must be billed directly to the hospital.

CPT Codes

CPT codes listed in this Directory are provided only as guidance to assist you in billing. CPT codes listed reflect our interpretation of CPT coding requirements and are subject to change at any time. It is the client's responsibility to verify the accuracy of the codes. A copy of the changes to CPT coding recommendations for 2005 precedes the test listing section. If you have any questions, please refer to the Current Procedural Terminology (CPT) manual published by the American Medical Association. To verify reimbursement, or if you have any questions regarding usage of a CPT code, please contact your local Medicare carrier.

Medical Necessity / Diagnosis Codes

Every third-party bill must have a valid diagnosis code. Please be sure to put the ICD-10 Code(s) applicable to the patient's condition for the specified date of service on the requisition in the box marked "Diagnostic Codes ICD-10". Medicare diagnosis codes must be coded to the highest level of specificity. Please refer to the International Classification of Diseases (ICD-10) manual, as well as the Medical Regulations and Manuals issued or authorized by the Center for Medicare and Medicaid Services (CMS) for diagnosis coding rules and regulations. If the claim is denied due to lack of medical necessity, KSL Beutner Laboratories will send a request for an additional ICD-10 code or other evidence of medical necessity directly to the ordering Doctor.

Specimen Requirements

SPECIMEN COLLECTION KITS ARE AVAILABLE FREE OF CHARGE FROM KSL BEUTNER LABORATORIES. CALL 1-800-288-0549 FOR AN IMMEDIATE SHIPMENT OF COLLECTION KITS.

General Requirements

- Biopsy Specimens for Direct immunofluorescence studies must be collected in Michel's medium (yellow top tube) or Zeus medium. Specimens inadvertently placed in formalin solution and then transferred into Michel's medium may compromise the results (J Am Acad of Dermatol 2011;65:106-11).
- **Biopsy Specimens for H&E should be placed in 10% formalin (blue top tube).**

Serum Studies

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Disease Specific Requirements

BULLOUS DISEASES: INDICATED DIRECT IMMUNOFLUORESCENT (DIF) AND SERUM STUDIES

SKIN LESIONS

If pemphigoid or epidermolysis bullosa acquisita (EBA), Laminin 332 pemphigoid, p200 pemphigoid, or bullous lupus erythematosus (LE) is suspected:

- Take skin biopsy with ~2/3 normal skin and ~1/3 edge of lesion
- For DIF on 1.0 M NaCl split skin to differentiate between pemphigoid and EBA, AECP and bullous LE, take second biopsy ~3mm from lesion
- Serum studies may also be indicated to determine the specific autoantibodies

If pemphigus is suspected:

- Take skin biopsy with ~2/3 normal skin and ~1/3 lesion edge plus serum for best diagnostic results
- Serum studies are also indicated for confirmation

If dermatitis herpetiformis is suspected,

- Take normal skin ~3 mm from lesion for best results
- Serum tests – EmA and tTG increase sensitivity
- Test for eTG antibodies may also be helpful in ruling out DH

If porphyria or pseudoporphyria is suspected:

- Take skin biopsy with ~2/3 normal skin and ~1/3 lesion edge for best DIF results

If in doubt:

- Take two biopsies — one perilesional, as for pemphigoid, and one normal, as for DH, for best results

If eruptions with other non-disease specific immune deposits are suspected, including lichen planus or lichenoid eruption or related disorders:

- Take biopsy as for porphyria for DIF and lesional biopsy for light microscopy

MUCOSAL LESIONS

If pemphigoid is suspected:

- Take normal mucosa ~3 mm from lesion or Nikolsky sign

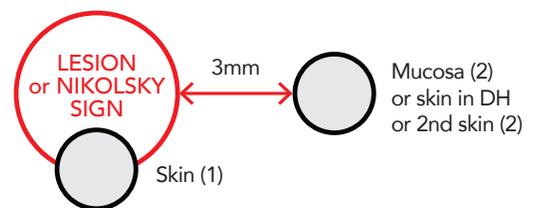
If pemphigus or paraneoplastic pemphigus is suspected:

- Take normal mucosa ~3 mm from lesion or Nikolsky sign plus serum

If erosive lichen planus (LP) is suspected:

- Take mucosal biopsy with ~2/3 normal mucosa and ~1/3 edge of lesion or of Nikolsky sign for best DIF results

BLISTERING AND OTHER ERUPTIONS



- (1) Skin biopsy in most pemphigus / pemphigoid cases.
- (2) Mucosal biopsy or skin biopsy for DH or 2nd skin biopsy for pemphigus or pemphigoid cases.

HEREDITARY EPIDERMOLYSIS BULLOSA (EB)

If hereditary EB needs to be classified or confirmed:

- Take biopsy of induced lesion in normal skin.

Preferred Skin Site:

- Normal appearing skin preferably upper inner arm just above the elbow.

SPECIAL METHOD OF OBTAINING SPECIMENS

An attempt should be made prior to biopsy to induce microscopic cleavage. The only possible exception would be in patients with generalized junctional or severe generalized recessive dystrophic EB, in whom there is such inherent mechanical skin fragility as to readily demonstrate cleavage planes just with the performance of routine punch biopsy technique. In general, it is suggested that the following be done prior to biopsy of such a skin area:

- The area to be biopsied should be sterilely prepped.
- Using the eraser (cleaned with an alcohol swab) part of a pencil, apply firm pressure downward and then laterally in a rotary fashion (approximately 180 degrees each way for 3-5 times).

If after lifting up the pencil eraser it is obvious that the skin has markedly split, then this procedure should be re-done a bit less vigorously (to at most demonstrate a minimal puckering of the skin) in an adjacent area. However, if absolutely no change is visible after having tried to induce a blister, then one should still biopsy that area in the hope that microscopic cleavage will be noted. For example, the latter is usually the case with localized EB simplex although we can still usually demonstrate cleavage in distant skin if it is pretreated in this manner. 4mm. punch biopsy should then be taken of this area and the entire specimen then placed into immunofluorescence transport medium (BL, Zeus or Michel's).

Under no circumstances should:

- Lesional tissue be sent since frequently it contains multiple artifactual as well as true cleavage planes, thereby making diagnosis very difficult. In addition, the presence of a blister usually is associated with the release of enzymes which tend to digest the skin proteins such that we cannot satisfactorily get antibodies to react with the specific antigens.
- Skin be obtained from the palms or soles, since the overall thickness of that tissue makes it very difficult to demonstrate skin cleavage or early blister formation.

References:

- Fine JD, Eady RAJ, Bauer EA et al. Revised classification system for inherited epidermolysis bullosa: Report of the second international consensus meeting on diagnosis and classification of epidermolysis bullosa. *J Am Acad Dermatol.* 2000; 42:1051-66.
- Fine JD, McGrath J, Eady RA. Inherited epidermolysis bullosa comes into the new millennium: A revised classification system based on current knowledge of pathogenetic mechanisms and the clinical, laboratory, and epidemiologic findings of large well-defined patient cohorts. *J Am Acad Dermatol.* 2000; 43:135-37.
- Fine JD, Eady RAJ, Bauer EA et al. The classification of inherited epidermolysis bullosa (EB): Report of the Third International Consensus Meeting on Diagnosis and Classification of EB. *J Am Acad Dermatol.* 2008; 58:931-50.

CONNECTIVE TISSUE DISEASES: INDICATED DIRECT IMMUNOFLUORESCENT (DIF) AND SERUM STUDIES

If SLE is suspected:

- Take biopsy of sun-exposed, normal skin of forearm for DIF for LE band test and blood in serum separator tube (SST)
- Serum can be sent in red top tube

If DLE is suspected:

- Take biopsy of untreated lesion of 3 or more months duration in sun-exposed area for DIF and for light microscopy
- Non-sun-exposed areas are of little value

If SCLE or Sjogren's syndrome is suspected:

- Take sun-exposed skin lesion biopsy for DIF for in vivo ANA and blood in SST for serum tests
- Serum can be sent in a red top tube in red top tube

If systemic sclerosis (SSc) is suspected:

- Take biopsy of sun-exposed skin for DIF studies and blood in SST for serum tests
- Serum can be sent in a red top tube in red top tube

IMMUNE COMPLEX MEDIATED VASCULITIS: INDICATED DIRECT IMMUNOFLUORESCENT STUDIES

If leukocytoclastic vasculitis is suspected, (or most other immune complex vasculitides):

- Take biopsy for DIF of a fresh lesion, less than 48 hours old
- DIF studies of older lesions (more than 72 hours old) yield low sensitivity
- Perilesional normal skin specimens have lower sensitivity for detection of immune complexes by DIF
- Light microscopic biopsy studies are also indicated

If Henoch Schoenlein purpura is suspected:

- Take biopsy of a fresh lesion (less than 48 hours old)
- Light microscopic biopsy studies are also indicated

If stasis dermatitis is suspected:

- Take biopsy for DIF of edge of skin lesion plus adjacent normal skin

Disease Specific Testing

BULLOUS DISEASES: BIOPSY STUDIES / SKIN AND MUCOSA

Direct Immunofluorescence (DIF)

KSL Beutner Test Code:

- #001

Routine Panel Tests for the Presence of:

- IgG, IgA, IgM, Fibrin, C3 and IgG4.

Note:

- IgG1 may be added at an additional charge, if greater sensitivity is required.

Methodology:

- Direct Immunofluorescence

Reference Range:

- Detailed interpretation accompanies report.

CPT Code:

- 88346 (x no of biopsies), 88350 (x immune stains)

Turnaround Time:

- Report availability is within 48-72 hours from the time of specimen receipt.

Biopsy Site Selection:

- Proper biopsy sites are dependent on the suspected diagnosis.

Specimen Requirements:

- The yellow capped tubes provided with KSL Beutner collection kits contain a holding solution for immunofluorescence specimens. A biopsy from a Lesional, Normal and/or Perilesional site should be placed in these collection tubes. If these tubes are unavailable, submit specimen in Zeus / Michel's fixative. Transport at room temperature.

Sample Stability:

- Room temperature = stable in appropriate solution for 10 days.

Differentiation of Bullous Pemphigoid from Epidermolysis Bullosa Acquisita by DIF of 1M NaCl split biopsy (with no vesicles)

KSL Beutner Test Code:

- #002

Methodology:

- Direct Immunofluorescence

Reference Range:

- Detailed interpretation accompanies report.

CPT Code:

- 88346 (x no of biopsies), 88350 (x immune stains)

Turnaround Time:

- Report availability is within 48-72 hours from the time of specimen receipt.

Biopsy Site Selection:

- Normal skin (3mm from a lesion). This test requires an intact epidermis and dermal-epidermal junction. If the initial DIF (test code#001) reveals epidermal separation, this test cannot be done.

Specimen Requirements:

- The yellow capped tubes provided with KSL Beutner collection kits contain a holding solution for immunofluorescence specimens. A biopsy from a Normal site should be placed in these collection tubes. If these tubes are unavailable, submit specimen in Zeus / Michel's fixative. Transport at room temperature.

Sample Stability:

- Room temperature = stable in appropriate solution for 10 days.

Light Microscopy

KSL Beutner Test Code:

- #003

Methodology:

- Histology processing, staining and Interpretation

Reference Range:

- Not applicable. Detailed interpretation accompanies report.

CPT Code:

- 88305

Turnaround Time:

- Report availability is within a week from the time of specimen receipt.

Specimen Requirements:

- Biopsies for H &E studies should be submitted in 10% neutral buffered formalin containers. Transport at room temperature.

Sample Stability:

- Room temperature = indefinitely.

LUPUS ERYTHEMATOSUS CONNECTIVE TISSUE AND VASCULAR DISEASE: BIOPSY STUDIES / SKIN AND MUCOSA

Direct Immunofluorescence (DIF) for LE (Systemic, Discoid, & Sub-Acute Cutaneous)

KSL Beutner Test Code:

- #005

Routine Panel Tests for the Presence of:

- IgG, IgA, IgM, Fibrin, and C3

Methodology:

- Direct Immunofluorescence

Reference Range:

- Not applicable, detailed interpretation accompanies report.

CPT Code:

- 88346 (x no of biopsies), 88350 x4

Turnaround Time:

- Report availability is within 48-72 hours from the time of specimen receipt.

Biopsy Site Selection:

- Proper biopsy sites are dependent on the suspected diagnosis.

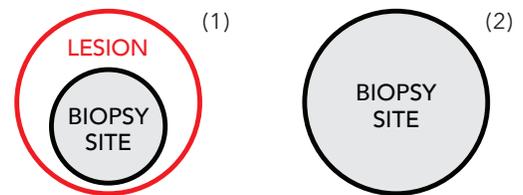
Specimen Requirements:

- The yellow capped tubes provided with KSL Beutner collection kits contain a holding solution for immunofluorescence specimens. A biopsy from a Lesional, Normal and/or Perilesional site should be placed in separate collection tubes. If these tubes are unavailable, submit specimen in Zeus / Michel's fixative. Transport at room temperature.

Sample Stability:

- Room temperature = stable in appropriate solution for 10 days

COLLAGEN VASCULAR DISEASES



(1) Sun Exposed skin biopsy in most LE cases.
Skin biopsy to rule out Henoch Schoenlein purpura and vasculitis (lesion less than 48 hours old)

(2) Skin biopsy to rule out SLE

Direct Immunofluorescence (DIF) for Vasculitis

KSL Beutner Test Code:

- #006

Routine Panel Tests for the Presence of:

- IgG, IgA, IgM, Fibrin, and C3

Methodology:

- Direct Immunofluorescence

Reference Range:

- Not applicable, detailed interpretation accompanies report.

CPT Code:

- 88346 (x no of biopsies), 88350 x4

Turnaround Time:

- Report availability is within 48-72 hours from the time of specimen receipt.

Biopsy Site Selection:

- Proper biopsy sites are dependent on the suspected diagnosis.

Specimen Requirements:

- The yellow capped tubes provided with KSL Beutner collection kits contain a holding solution for immunofluorescence specimens. A biopsy from a Lesional site (lesion <48 hours old) should be placed in these collection tubes. If these tubes are unavailable, submit specimen in Zeus / Michel's fixative. Transport at room temperature.

Sample Stability:

- Room temperature = stable in appropriate solution for 10 days

Direct Immunofluorescence (DIF) for Dermatomyositis

KSL Beutner Test Code:

- #007

Routine Panel Tests for the Presence of:

- IgG, IgA, IgM, Fibrin, C3, and C5b-9

Methodology:

- Direct Immunofluorescence

Reference Range:

- Not applicable, detailed interpretation accompanies report.

CPT Code:

- 88346 (x no of biopsies), 88350 x5

Turnaround Time:

- Report availability is within 48-72 hours from the time of specimen receipt.

Biopsy Site Selection:

- Proper biopsy sites are dependent on the suspected diagnosis.

Specimen Requirements:

- The yellow capped tubes provided with KSL Beutner collection kits contain a holding solution for immunofluorescence specimens. A biopsy from a Lesional site should be placed in these collection tubes. If these tubes are unavailable, submit specimen in Zeus / Michel's fixative. Transport at room temperature.

Sample Stability:

- Room temperature = stable in appropriate solution for 10 days

IF Mapping for Hereditary Epidermolysis Bullosa

KSL Beutner Test Code:

- #011

Routine Panel Tests:

- Type VII Collagen, Type IV Collagen, Keratin 14, Laminin 332, Plectin, Alpha 6 integrin, Beta 4 integrin, Type XVII Collagen. If needed, additional tests may be done.

Methodology:

- Direct Immunofluorescence

Reference range:

- Not applicable, detailed interpretation accompanies report.

CPT Code:

- 88346 (x no of biopsies), 88350 x7-9

Turnaround Time:

- Report availability is within 3-4 days from the time of specimen receipt.

Biopsy Site and Specimen Requirements:

- A skin biopsy of an induced micro-vesicle on normal skin is recommended. Normal appearing skin preferably upper inner arm just above the elbow should be selected for inducing a lesion. The yellow capped tubes provided with Beutner collection kits contain a holding solution for IF mapping. If these tubes are unavailable, submit specimen in Zeus / Michel's fixative. Transport at room temperature.

Special Method of Obtaining Specimens:

- An attempt should be made prior to biopsy to induce microscopic cleavage. The only possible exception would be in patients with generalized junctional or severe generalized recessive dystrophic EB, in whom there is such inherent mechanical skin fragility as to readily demonstrate cleavage planes just with the performance of routine punch biopsy technique. In general, it is suggested that the following be done prior to biopsy of such a skin area:
 - The area to be biopsied should be sterilely prepped and then circled.
 - Using the eraser (cleaned with an alcohol swab) part of a pencil, apply firm pressure downward and then laterally in a rotary fashion (approximately 180 degrees each way for 3-5 times).
 - If after lifting the pencil eraser it is obvious that the skin has markedly split, then this procedure should be re-done a bit less vigorously (to at most demonstrate a minimal puckering of the skin) in an adjacent area. However, if absolutely no change is visible after having tried to induce a blister, then one should wait for 15-20 minutes and then biopsy that area in the hope that microscopic cleavage may be noted. 4mm. punch biopsy should then be taken of this area and the entire specimen then placed into immunofluorescence transport medium (BL, Zeus or Michel's).

UNDER NO CIRCUMSTANCES SHOULD:

- Lesional tissue be sent since it frequently contains multiple artifactual as well as true cleavage planes, thereby making diagnosis very difficult. In addition, the presence of a blister usually is associated with the release of enzymes which tend to digest the skin proteins such that we cannot satisfactorily get antibodies to react with the specific antigens.
- Skin be obtained from the palms or soles, as the overall thickness of that tissue makes it very difficult to demonstrate skin cleavage or early blister formation.

Sample Stability:

- Room temperature = stable in appropriate solution for 5 days.

Clinical Relevance:

- Immunofluorescence (IF) mapping of a skin biopsy taken after inducing a micro-vesicle on normal skin is recommended as a primary diagnostic test for inherited EB. Reactions of antigens along the clefts helps in differentiating EB simplex, junctional EB and dystrophic EB by localizing the plane of cleavage above or below the lamina densa or in the epidermis. The absence of reactions or weak reactions with specific antibodies can point to defective proteins associated with certain forms of inherited EB.
- The biopsy specimen for IF mapping studies is processed in two steps:
 - 1. Primary Screening:**
Primary screening for collagen types IV and VII and keratin 14 for IF mapping and biopsy suitability.
 - 2. Extended IF Mapping:**
For suitable biopsies, tests for plectin, alpha 6 integrin, beta 4 integrin, laminin 332, type XVII collagen, and possibly keratin 5 (if EBS) and beta 3 laminin.
- The interpretations of this test should be correlated and confirmed with electron microscopy and studies for gene mutations.

BULLOUS DISEASES: SERUM STUDIES

Pemphigus / Pemphigoid Antibody Titer – IgG & IgG4

KSL Beutner Test Code:

- #013

Methodology:

- Indirect Immunofluorescence

Substrate:

- Primate Esophagus

Reference Range:

- Negative: <1:10

Units:

- Titer

CPT Code:

- 88346, 88350

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- The detection of anti-skin (IC and BMZ) antibodies aids in the diagnosis, and sometimes prognosis, of autoimmune bullous diseases including pemphigus, pemphigoid, cicatricial pemphigoid, and epidermolysis bullosa acquisita (EBA). Epithelial intercellular antibodies are diagnostic for pemphigus and occur in over 90% of patients with active forms. Antibodies to basement membrane antigens of stratified squamous epithelium occur in about 70% of active bullous pemphigoid, 50% of EBA and 10% of cicatricial pemphigoid patients.

Differentiation of Bullous Pemphigoid from Epidermolysis Bullosa Acquisita (EBA) on Split Skin – IgG & IgG4

KSL Beutner Test Code:

- #014

Methodology:

- Indirect Immunofluorescence

Substrate:

- Split Human Skin

Reference Range:

- Negative: <1:5

Units:

- Titer

CPT Code:

- 88346, 88350

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Epidermolysis bullosa acquisita (EBA) can mimic bullous pemphigoid (BP) clinically, histologically and immunologically. In this indirect immunofluorescence assay, EBA and BP antibodies can be distinguished by their localization in skin that is split at the lamina lucida. This test and added follow up tests help differentiate EBA / bullous LE / anti-epiligrin cicatricial pemphigoid from bullous pemphigoid.

Desmoglein (Dsg) 1 & Dsg 3 Antibodies

KSL Beutner Test Code:

- #015

Methodology:

- ELISA

Reference Range Values:

Dsg1 Ratio

Negative: <1

Positive ≥1

Dsg3 Ratio

Negative: <1

Positive: ≥1

Units:

- Units/mL

CPT Code:

- 83516 x2

Schedule / Turnaround Time:

- Assay performed once every week. Report availability is within one week from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Pemphigus includes a group of often fatal autoimmune blistering diseases characterized by intraepidermal and/or intraepithelial lesions. Pemphigus vulgaris and its variants may present with oral or other mucosal lesions alone or with mucosal plus skin lesions. Pemphigus foliaceus and its variants present with skin lesions alone. Indirect Immunofluorescence studies reveal that both forms of pemphigus are caused by autoantibodies to cell surface antigens of stratified epithelia of mucous membranes and epidermal layer of the skin. These antibodies bind to calcium dependent adhesion molecules in cell surface desmosomes, notably desmoglein 1(DSG-1) in pemphigus foliaceus and desmoglein 3 (DSG-3) in pemphigus vulgaris. Pemphigus vulgaris patients with both mucosal and skin lesions have antibodies to both DSG-3 and DSG-1. The diagnosis of pemphigus depends on biopsy and serum studies that characterize lesions and detect the autoantibodies that cause them. Serum studies afford highly sensitive diagnostic aids. The identification of the reactive antigens as DSG-1 and DSG-3 has made it possible to develop highly specific and sensitive ELISA methods.

Bullous Pemphigoid (BPAG2) 180 & BP (BPAG1) 230 Antibodies

KSL Beutner Test Code:

- #016

Methodology:

- ELISA

Reference Range:

- Ratio <1.0: Negative
- Ratio ≥1.0: Positive

Units:

- Ratio

CPT Code:

- 83516 x2

Schedule / Turnaround Time:

- Assay performed once every week. Report availability is within one week from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Bullous pemphigoid (BP) is an autoimmune mediated immunobullous skin disorder found mainly in the elderly population and is characterized by frequent occurring of tense blisters and erythema. Antibodies are directed to the basement membrane zone and are frequently found in the serum of patients. Target antigens of the autoantibodies in BP patient serum are BP230 and BP180, also called BPAG1 and BPAG2.
- Molecular weight of these antigens is 230 kD and 180 kD respectively. BP180 is thought to be the direct target of the autoantibody because of its location, and the autoantibodies against BP230 are thought to be secondarily produced. The antibodies against BP180 are thought to be pathogenic, because the rabbit antibody against mouse in the NC16a region of BP180 forms blisters similar to BP when injected into neonatal mice. The main epitope of BP180 is located in the region close to cell membrane called NC16a and most patient serum reacts with the recombinant NC16a protein. Serum levels of BP180 co-relate with the disease activity.

Laminin 332 Antibodies

KSL Beutner Test Code:

- #010

Methodology:

- Indirect Immunofluorescence

Reference Range:

- Negative < 1:10

Units:

- Titer

CPT Code:

- 88346, 88350

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt. The turnaround time may be more than 72 hours if there is a need to run this test in batches.

Specimen Requirements:

- Specimen need not be refrigerated or frozen. Collect 5-10 ml of blood in a red top or serum separator tube. Minimum volume requested is 2ml. If possible, separate serum from clot and place into red top tube provided with Beutner Laboratories collection kits. If separation facilities are not available, the blood can be sent in the tube used for collection.

Sample Stability:

- Room temperature = 1 week; 2°C to 8°C = 2 weeks; -25°C to -15°C = 1 year

Clinical Relevance:

- Mucous membrane pemphigoid (MMP) is a mucosal autoimmune subepithelial blistering disease mediated autoantibodies against various autoantigens in the basement membrane zone (BMZ). The predominant autoantigens are BP180, BP230, laminin 332, integrin alpha 6 beta 4, and collagen VII (COL7) (Egan, 2001). MMP mediated by antibodies to laminin 332 presents with a different clinical presentation and risk. Anti-laminin 332 MMP lesions tend to scar and can result in serious complications depending on the mucosal surface affected. Scarring of ocular mucosa can result in symblepharon, disruption of the tear film, entropion, and/or corneal opacities, all of which may lead to blindness. Other irreversible, severe complications are esophageal, anal stenosis, and urethral strictures. An additional complication of anti-laminin 332 MMP increased relative risk of solid epithelial malignancies, mainly adenocarcinoma affecting the gastrointestinal, genital mucosa, and lungs (Balestri, 2016). Clinically, patients with anti-laminin 332 MMP cannot be differentiated from patients with MMP with autoantibodies against the other main target antigen, BP180, although pharyngeal involvement was reported to occur less frequently in the latter patients. Patients with anti-laminin 332 MMP cannot be differentiated from other variants of MMP based on clinical appearance and can only be distinguished by serological testing. Identification of MMP patients with laminin 332-specific antibodies are essential since 25%-30% of these patients might have a malignancy. The introduction of the laminin 332-specific biochip mosaic developed in 2019 by Goletz et al., provided a widely available standardized test system for the detection of anti-laminin 332 serum autoantibodies. External and internal studies showed that the test showed a sensitivity of 84% and a specificity of 99.8%.

References:

Balestri R, Magnano M, La Placa M, Patrizi A, Angileri L, Tengattini V, Bardazzi F. Malignancies in bullous pemphigoid: a controversial association. *J Dermatol* 2016; 43: 125-133.

Egan CA, Lazarova Z, Darling TN, Yee C, Cote T, Yancey KB. Anti-epiligrin cicatricial pemphigoid and relative risk for cancer. *Lancet* 2001; 9: 1850-1851.

Goletz S, Probst C, Komorowski L, Schlumberger W, Fechner K, van Beek N, et al. A Sensitive and Specific Assay for the Serological Diagnosis of Antilaminin 332 Mucous Membrane Pemphigoid. *Br J Dermatol* (2019) 180(1):149-56. doi: 10.1111/bjd.17202

Laminin Beta 4 (p200) antibodies

KSL Beutner Test Code:

- #012

Methodology:

- Indirect Immunofluorescence

Reference Ranges:

- Negative: < 1:10
Positive: ≥ 1:10

Units:

- Titer

CPT Code:

- 88346, 88350

Schedule / Turnaround Time:

- Assay performed once weekly.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment for up to 1 week
2-8°C = 2 weeks
-25 to -15°C = good for one freeze/thaw cycle

Clinical Relevance:

- P-200 pemphigoid is a rare sub-epidermal blistering disease first described by Zillikens in 1996. This novel disease is defined by tissue-bound immunoglobulins and/or complement C3 along the basement membrane zone (BMZ) and serum autoantibodies targeting a 200- kDa protein localized within the lower lamina lucida of the BMZ. Laminin γ 1 has been described as the 200 kDa target antigen recognized by 70% to 90% of patients. Failure of anti-laminin γ 1 IgG to show pathogenic effects in vitro and in vivo point to the presence of another autoantigen in anti-p200 pemphigoid. Recently, laminin β 4 (LAMB 4) has been identified as additional target antigen of anti-p200 pemphigoid. Clinically, p-200 pemphigoid may mimic bullous pemphigoid (BP) or other bullous autoimmune diseases. The clinical presentation can be like BP (approx. in 66% patients), linear IgA bullous dermatosis, epidermolysis bullosa acquisita (EBA), dermatitis herpetiformis and mucous membrane pemphigoid. Patients present with tense blisters, erosions and urticarial plaques, scars and/or milia can be present. The extremities (hands and feet) are most frequently involved followed by the trunk, and palmoplantar and cephalic involvement. Psoriasis is the most common coexisting dermatosis and it may precede anti-p200 pemphigoid.

Paraneoplastic Pemphigus Antibody Titer

KSL Beutner Test Code:

- #017

Methodology:

- Indirect Immunofluorescence

Substrate:

- Rat bladder

Reference Range:

- Negative: <1:10

Units:

- Titer

CPT Code:

- 88346, 88350

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Paraneoplastic pemphigus, or PNP, is a rare but often fatal autoimmune blistering disease, with five-year mortality rates approaching 40%. PNP is most commonly associated diagnoses are hematologic malignancies and occasionally sarcomas and other solid-organ malignancies. In children, the most commonly associated cancer is Castleman disease. Indirect immunofluorescence using rat bladder epithelium substrate is a sensitive and specific test, and it is positive in about 80% of cases of PNP. Paraneoplastic pemphigus, or PNP, is a rare but often fatal autoimmune blistering disease, with five-year mortality rates approaching 40%.

Pemphigus Antibody Titer Prognostic Test – IgG & IgG4 (Compares Old & New Sera)

KSL Beutner Test Code:

- #018

Methodology:

- Indirect Immunofluorescence

Substrate:

- Primate Esophagus

Reference Range:

- Negative: <1:10

Units:

- Titer

CPT Code:

- 88346, 88350

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- The comparison of the titers of anti-skin (IC) antibodies in old and new sera aid in diagnosis and prognosis of pemphigus patients.

Envoplakin Antibody

KSL Beutner Test Code:

- #009

Methodology:

- ELISA

Reference Range:

- Ratio <1.0 Negative
- Ratio >1.0 Positive

Units:

- Ratio

CPT Code:

- 83516

Schedule / Turnaround Time:

- Assay performed once weekly. Report availability is one week from the time of specimen receipt. Please call the laboratory if expedited results are needed.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Paraneoplastic pemphigus (PNP) is a life-threatening form of pemphigus that is associated with a neoplasm (e.g., non-Hodgkin's lymphoma, chronic lymphocytic leukemia, Castleman tumor, thymoma, sarcoma, Waldenstrom's macroglobulinemia). Pathogenesis is based on a combination of humoral and cellular autoimmune responses. Circulating autoantibodies are directed against multiple antigens, including predominantly plakins (envoplakin, periplakin, desmoplakin I, desmoplakin II, epiplakin), plectin, Dsg1, Dsg3 and BP230. Due to their high specificity (91–100%), anti-envoplakin autoantibodies are considered an important diagnostic marker for paraneoplastic pemphigus. This ELISA is 72% sensitive in detecting antibodies to envoplakin in PNP patients.

Collagen VII Antibodies ELISA

KSL Beutner Test Code:

- #023

Methodology:

- ELISA

Reference Range:

- <6.0 U/mL Negative
- ≥6.0 U/mL Positive

Units:

- U/mL

CPT Code:

- 83516

Schedule / Turnaround Time:

- Assay performed once weekly. Report availability is one week from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Type VII Collagen is found in the lamina densa and the sublamina densa fibrillar area of the dermal-epidermal junction. The pathogenicity of autoantibodies targeting collagen VII has been independently demonstrated both in vitro, ex vivo and in vivo. Collagen VII autoantibodies specific to COL7 is the current standard for EBA diagnosis (Hashimoto, 2012). Type VII collagen (COL7)-specific autoantibodies were primarily of the IgG isotype and are found in over 75% of the EBA patients (Iwata, 2018). The immunodominant domains of type VII collagen can also be recognized by IgG autoantibodies from patients with bullous lupus erythematosus (BSLE). Anti-type VII collagen autoantibodies can be detected 3 months before the inception of BSLE in patients with SLE. In a study by Grabell (2015) of BSLE, 100% of the patients had circulating Collagen VII antibodies before and during the skin eruption. The healthy and SLE controls did not show any circulating collagen VII antibodies (Grabell, 2015, Ishikawa, 1997). This ELISA (MBL) detects antibodies to autoreactive non-collagenous 1 and 2 (NC1 and NC2) domains of type VII collagen. In a study on suspected patients with EBA / bullous LE by Bain E et al., this test gave 87.5% sensitivity and 100% specificity. Anti-type VII collagen autoantibodies can be detected 3 months before the inception of BSLE in patients with SLE. In a study by Grabell (2015) of BSLE, 100% of the patients had circulating Collagen VII antibodies before and during the skin eruption. The healthy and LSE controls did not show any circulating collagen VII antibodies (Grabell, 2015, Ishikawa, 1997).

Selected Reference:

Hashimoto T, Ishii N, Ohata C, Furumura M. Pathogenesis of epidermolysis bullosa acquisita, an autoimmune subepidermal bullous disease. *J Pathol.* 2012;228:1-7

Iwata, H., Vorobyev, A., Koga, H., Recke, A., Zillikens, D., Prost-Squarcioni, C., ... & Ludwig, R. J. (2018). Meta-analysis of the clinical and immunopathological characteristics and treatment outcomes in epidermolysis bullosa acquisita patients. *Orphanet journal of rare diseases*, 13(1), 153.

Grabell, D. A., Matthews, L. A., Yancey, K. B., & Chong, B. F. (2015). Detection of type VII collagen autoantibodies before the onset of bullous systemic lupus erythematosus. *JAMA dermatology*, 151(5), 539-543.

Ishikawa O, Zaw KK, Miyachi Y, Hashimoto T, Tanaka T. (1997). The presence of anti-basement membrane zone antibodies in the sera of patients with non-bullous lupus erythematosus. *Br J Dermatol.* 136(2):222-226.

Bain EE, Grover RK, Plunkett RW, Beutner EH. Bain EE, Grover RK, Plunkett RW, Beutner EH. Detection of collagen VII autoantibodies to NC1 and NC2 domains of collagen VII by ELISA in suspected epidermolysis bullosa acquisita and bullous lupus erythematosus patients. *J Dermatol Sci.* 2012 Feb;65(2):155-6.

Linear IgA Bullous Dermatitis (LABD), Chronic Bullous Dermatitis of Childhood (CBDC) Antibody Titer – IgA

KSL Beutner Test Code:

- #024

Methodology:

- Indirect Immunofluorescence

Substrate:

- Normal human skin and Split Human skin

Reference Range:

- Negative: <1:5

Units:

- Titer

CPT Code:

- 88346, 88350

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Linear IgA Bullous Dermatitis (LABD) is a chronic, acquired, autoimmune blistering disease. It is characterized by subepidermal blistering and linear deposition of immunoglobulin A (IgA) basement membrane antibodies. The disease affects both children and adults. There are some differences in their clinical presentations, therefore the disease in children is termed as Chronic Bullous Disease of Childhood (CBDC). The immunopathology of LABD and CBDC is similar (Venning, 2011). IgA antibodies to BMZ of skin by IIF on split human skin can be detected in about 30% of adult patients with LABD and in almost 75% of children with CBDC. This test helps to differentiate the autoantibodies reacting on the epidermal roof of the split human skin (target antigens are the shed ectodomains of BP180 molecule) from the antibodies reacting on the floor (target antigen is LAD285 and in rare cases type VII Collagen). Patients with LABD may have co-existing IgG antibodies, therefore IIF test for IgG and IgG4 antibodies on Split Human Skin (#014) should also be done.

Selected Reference:

Vanessa A. Venning. Linear IgA Disease: Clinical Presentation, Diagnosis, and Pathogenesis. *Dermatologic Clinics*, 2011;29 (3): 453-458.

Pemphigus

KSL Beutner Test Code:

- #025 Includes Tests: #013 Pemphigus / Pemphigoid antibody titer – IgG & IgG4
#015 Desmoglein (DSG) 3 & DSG 1 Antibodies

Methodology:

- Indirect Immunofluorescence and ELISA

Substrate:

- Primate Esophagus / ELISA plate

Reference Range:

- Please see individual tests

CPT Code:

- Please see individual tests

Schedule / Turnaround Time:

- Please see individual tests

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Paraneoplastic Pemphigus

KSL Beutner Test Code:

- #026 Includes Tests: #013 Pemphigus / Pemphigoid antibody titer – IgG & IgG4
#015 Desmoglein (DSG) 3 & DSG 1 Antibodies
#017 Paraneoplastic Pemphigus Antibody Titer – IIF on Rat Bladder
#009 Envoplakin ELISA Alpha 2 Macroglobulin Like Protein 1 (A2ML1) Immunoblot
#065 Alpha 2 Macroglobulin Like Protein 1 (A2ML1) Immunoblot

Methodology:

- Indirect Immunofluorescence, ELISA and Immunoblot

Substrate:

- Primate Esophagus / ELISA Plate / Rat Bladder / Immunoblot Test Strips

Reference Range:

- Please see individual tests

CPT Code:

- Please see individual tests

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Please see individual tests.

Pemphigoid

KSL Beutner Test Code:

- #027 Includes Tests: #013 Pemphigus / Pemphigoid antibody titer – IgG & IgG4
#014 Differentiation of Bullous Pemphigoid from EBA on Split Skin – IgG & IgG4
#016 BP230 & BP180 Antibodies – ELISA

Methodology:

- Indirect Immunofluorescence and ELISA

Substrate:

- Primate Esophagus / Human Split Skin / ELISA plate

Reference Range:

- Please see individual tests

CPT Code:

- Please see individual tests

Schedule / Turnaround Time:

- Please see individual tests

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

EBA / Bullous LE

KSL Beutner Test Code:

- #061 Includes Tests: #013 Pemphigus / Pemphigoid antibody titer – IgG & IgG4
#014 Split Skin IgG & IgG4
#023 Collagen VII Antibody ELISA

Methodology:

- Indirect Immunofluorescence and ELISA

Reference Ranges:

- Monkey Esophagus (IgG/IgG4)
Negative < 10
- Split Skin (IgG/IgG4)
Negative < 5
- Type VII Collagen
Negative < 6.0 U/mL
Positive ≥ 6.0 U/mL

Units:

- Titer, U/mL

CPT Code:

- 88346 (x2), 88350 (x2);83520 (x1)

Schedule / Turnaround Time:

- See individual tests

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- See individual tests

Laminin 332 Pemphigoid

KSL Beutner Test Code:

- #062 Includes Tests: #013 Pemphigus / Pemphigoid antibody titer – IgG & IgG4
#014 Split Skin IgG & IgG4
#010 Laminin 332 Antibody

Methodology:

- Indirect Immunofluorescence

Reference Ranges:

- Monkey Esophagus (IgG/IgG4)
Negative < 10
- Split Skin (IgG/IgG4)
Negative < 5
- Laminin 332 Transfected Cells
Negative < 10

Units:

- Titer

CPT Code:

- 88346 (x3), 88350 (x2)

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt. The turnaround time may be more than 72 hours if there is a need to run this test in batches.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment; 2-8°C = 5 days; -25°C to -15°C = 1 year

Clinical Relevance:

- Mucous membrane pemphigoid (MMP) is a mucosal autoimmune subepithelial blistering disease mediated autoantibodies against various autoantigens in the basement membrane zone (BMZ). The predominant autoantigens are BP180, BP230, laminin 332, integrin alpha 6 beta 4, and collagen VII (COL7) (Egan, 2001). MMP mediated by antibodies to laminin 332 presents with a different clinical presentation and risk. Anti-laminin 332 MMP lesions tend to scar and can result in serious complications depending on the mucosal surface affected. Scarring of ocular mucosa can result in symblepharon, disruption of the tear film, entropion, and/or corneal opacities, all of which may lead to blindness. Other irreversible, severe complications are esophageal, anal stenosis, and urethral strictures. An additional complication of anti-laminin 332 MMP increased relative risk of solid epithelial malignancies, mainly adenocarcinoma affecting the gastrointestinal, genital mucosa, and lungs (Balestri, 2016). Clinically, patients with anti-laminin 332 MMP cannot be differentiated from patients with MMP with autoantibodies against the other main target antigen, BP180, although pharyngeal involvement was reported to occur less frequently in the latter patients. Patients with anti-laminin 332 MMP cannot be differentiated from other variants of MMP based on clinical appearance and can only be distinguished by serological testing. Identification of MMP patients with laminin 332-specific antibodies are essential since 25%-30% of these patients might have a malignancy. The introduction of the laminin 332-specific biochip mosaic developed in 2019 by Goletz et al., provided a widely available standardized test system for the detection of anti-laminin 332 serum autoantibodies. External and internal studies showed that the test showed a sensitivity of 84% and a specificity of 99.8%.

Selected Reference:

Balestri R, Magnano M, La Placa M, Patrizi A, Angileri L, Tengattini V, Bardazzi F. Malignancies in bullous pemphigoid: a controversial association. *J Dermatol* 2016; 43: 125-133.

Egan CA, Lazarova Z, Darling TN, Yee C, Cote T, Yancey KB. Anti-epiligrin cicatricial pemphigoid and relative risk for cancer. *Lancet* 2001; 9: 1850-1851.

Goletz S, Probst C, Komorowski L, Schlumberger W, Fechner K, van Beek N, et al. A Sensitive and Specific Assay for the Serological Diagnosis of Antilaminin 332 Mucous Membrane Pemphigoid. *Br J Dermatol* (2019) 180(1):149-56. doi: 10.1111/bjd.17202

Laminin Beta 4 (p200) Pemphigoid

KSL Beutner Test Code:

- #063 Includes Tests: #013 Monkey Esophagus
#014 Split Skin for IgG, IgG4
#012 Laminin Beta 4 (p200) antibodies (IIF)

Methodology:

- Indirect Immunofluorescence

Reference Ranges:

- Laminin Beta 4 (p200) Antibodies (IIF)
Negative: < 1:10
Positive: ≥ 1:10
- Pemphigoid Antibody Titer IgG & IgG4
Negative: < 1:10
Positive: ≥ 1:10
- Split Skin IgG & IgG4
Negative: <1:5
Positive: ≥ 1:5

Units:

- Titer

CPT Code:

- 88346 x3, 88350 x3

Schedule / Turnaround Time:

- Please see individual tests.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment for up to 1 week
- 2°C to 8°C = 2 weeks
- -25°C to -15°C = good for one freeze/thaw cycle

Clinical Relevance:

- P-200 pemphigoid is a rare sub-epidermal blistering disease first described by Zillikens in 1996. This novel disease is defined by tissue-bound immunoglobulins and/or complement C3 along the basement membrane zone (BMZ) and serum autoantibodies targeting a 200- kDa protein localized within the lower lamina lucida of the BMZ. Laminin γ 1 has been described as the 200 kDa target antigen recognized by 70% to 90% of patients. Failure of anti-laminin γ 1 IgG to show pathogenic effects in vitro and in vivo point to the presence of another autoantigen in anti-p200 pemphigoid. Recently, laminin β 4 (LAMB 4) has been identified as additional target antigen of anti-p200 pemphigoid. Clinically, p-200 pemphigoid may mimic bullous pemphigoid (BP) or other bullous autoimmune diseases. The clinical presentation can be like BP (approx. in 66% patients), linear IgA bullous dermatosis, epidermolysis bullosa acquisita (EBA), dermatitis herpetiformis and mucous membrane pemphigoid. Patients present with tense blisters, erosions and urticarial plaques, scars and/or milia can be present. The extremities (hands and feet) are most frequently involved followed by the trunk, and palmoplantar and cephalic involvement. Psoriasis is the most common coexisting dermatosis and it may precede anti-p200 pemphigoid.

DH / CELIAC DISEASE / GLUTEN SENSITIVE ENTEROPATHY: SERUM PANELS

Endomysial (EmA) Antibody IgA & IgG Antibody

KSL Beutner Test Code:

- #020

Methodology:

- Indirect Immunofluorescence

Substrate:

- Primate Smooth Muscle (Primate Lower Esophagus)

Reference Range:

- Negative: <1:2.5

Units:

- Titer

CPT Code:

- 86231 x2

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- The detection of endomysial antibodies (EmA) aids in the diagnosis of gluten sensitive enteropathy, i.e. celiac disease (CD) and dermatitis herpetiformis (DH). Patients with CD and DH are reported to have antibodies to endomysium, reticulin and gliadin. Of the various antibody markers of CD and DH, EmA of the IgA class seem to be the most sensitive and specific for the diagnosis of DH. Tests for IgA TG2 (tTG or tissue transglutaminase) and for TG3 (eTG or epidermal transglutaminase) by ELISA should also be done for greater sensitivity.

IgA Epidermal Transglutaminase Antibody

KSL Beutner Test Code:

- # 022

Methodology:

- ELISA

Reference Range:

- <16 AU/mL Negative
- 16-22 AU/mL Grey range
- >22 AU/mL Positive

Units:

- AU/mL

CPT Code:

- 83516

Schedule / Turnaround Time:

- Assay performed once every two weeks. Report availability is two weeks from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Patients with Dermatitis Herpetiformis (DH) have gluten induced circulating IgA autoantibodies to tissue transglutaminase (TG2) and epidermal transglutaminase (TG3). Recent data indicated that DH is IgA-TG3 immunocomplex mediated disease developing in some gluten sensitive enteropathy (GSE) patients. There is pathogenic TG3 deposition in the papillary dermis and small blood vessels in skin of patients, colocalized with granular IgA deposits. IgA epidermal transglutaminase antibodies ELISA is a sensitive test for initial diagnosis and follow up of patients with DH.

Tissue Transglutaminase Antibody IgA & IgG

KSL Beutner Test Code:

- #053

Methodology:

- ELISA

Reference Range:

- IgA
<4 Units..... Negative
4-10 Units Equivocal
>10 Units..... Positive
- IgG
<6 Units..... Negative
6-9 Units Equivocal
>9 Units..... Positive

Units:

- Units/mL

CPT Code:

- 83516 x2

Schedule / Turnaround Time:

- Assay performed once weekly.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- IgA anti-endomysium antibodies (EmA), IgA and IgG anti-deamidated gliadin peptides (DGP), and IgA anti-tissue transglutaminase (tTG) antibodies are considered specific and sensitive serologic markers for gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis (DH). Although the IgA isotype of these antibodies usually predominates in celiac disease and dermatitis herpetiformis, individuals may also produce IgG isotypes, particularly if the individual is IgA deficient. The sensitivity and specificity of commercially available IgA tTG ELISA tests for the diagnosis of DH have been reported to range from 42% to 99% and 92% to 100%, respectively.

Deamidated Gliadin Antibody IgA & IgG

KSL Beutner Test Code:

- #054

Methodology:

- ELISA

Reference Range (IgA & IgG):

- <20 Units..... Negative
- 20-30 Units Equivocal
- >30 Units..... Positive

Units:

- Units/mL

CPT Code:

- 83516 x2

Schedule / Turnaround Time:

- Assay performed once weekly. Report availability is within one week from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- The diagnosis of celiac disease (CD) is based on mucosal changes detected by duodenal biopsy and positive serological tests (anti-tTG antibodies, anti-endomysium antibodies (EmA), and deamidated gliadin peptide (DGP) antibodies). Test for anti-deamidated peptides of gliadin (DGP) have replaced classic anti-native gliadin (AGA) tests. DGP IgG assays have a considerably higher diagnostic accuracy than AGA assays and can replace anti-tTG tests in patients with selective IgA deficiency. The combination of IgG anti-DGP plus IgA anti-tTG assays show greater sensitivity than a single test, with very high specificity. IgG DGP are particularly useful in identifying CD in early childhood (age < 2 years). The reported sensitivity and specificity of Anti-Gliadin DP antibodies in celiac disease is 84.4% and 98.5%. The PPV and NPV have been reported as 98.2% and 86.8% respectively. However, the definitive diagnosis of CD should be based on clinical, molecular, and histopathological findings.

Comprehensive DH / Celiac Disease Panel

KSL Beutner Test Code:

- #055 Includes Tests: #020 IgA & IgG Endomysial antibodies
#022 IgA Epidermal Transglutaminase Antibodies-ELISA
#053 Tissue Transglutaminase: IgA & IgG
#054 Deamidated Gliadin IgA & IgG

Methodology:

- Indirect Immunofluorescence, ELISA

Reference Range:

Endomysial EMA(IgG/IgA) (Titer)

- Negative <2.5

Epidermal transglutaminase eTG (IgA) (AU/mL)

- Negative <16
- Grey range 16-22
- Positive >22

Tissue Transglutaminase tTG (IgA) (U/mL)

- Negative <4
- Equivocal 4-10
- Positive >10

Tissue Transglutaminase tTG (IgG) (U/mL)

- Negative <6
- Equivocal 6-9
- Positive >9

Units:

- Titer, Units/mL

Deamidated Gliadin DGP (IgG/IgA) (U/mL)

- Negative <7
- Equivocal 7-10
- Positive >10

CPT Code:

- 86231 x2, 83516 x3, 86364 x2

Schedule / Turnaround Time:

- Assay performed once weekly.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- Please see individual tests.

CHRONIC ULCERATIVE STOMATITIS

Stratified Epithelium Specific Antinuclear Antibody (SES-ANA) for Chronic Ulcerative Stomatitis

KSL Beutner Test Code:

- #033

Methodology:

- Indirect Immunofluorescence

Substrate:

- Primate Esophagus

Reference Range:

- Based upon selective reactions on substrates used in the differential assay.

Units:

- Titer

CPT Code:

- 88346 (for initial SES-ANA Screen); if positive, 88350 (for establishing end point titer), 86038 (HEp-2 Screen), and 86039 (if applicable, HEp-2 Pattern).

Schedule / Turnaround Time:

- Assay performed daily Monday-Friday. Report availability is within 48-72 hours from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
2-8°C = 5 days
-25 to -15°C = 1 year

Clinical Relevance:

- The detection of stratified epithelium specific antinuclear antibodies assists in the diagnosis of connective tissue disease, and/or chronic ulcerative stomatitis.

Antinuclear Antibodies (ANA)

KSL Beutner Test Code:

- #042

Methodology:

- Indirect Immunofluorescence

Reference Ranges:

- Negative <1:40

Units:

- Titer

CPT Code:

- 86038, 86039, 86255

Schedule / Turnaround Time:

- Assay performed once per week.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.

Sample Stability:

- Room temperature = stable during shipment
- 2°C to 8°C = 5 days
- -25°C to -15°C = 1 year

Clinical Relevance

- Antinuclear antibodies (ANAs) are a diverse group of autoantibodies that recognize nuclear macromolecules and their complexes. ANAs are associated with various rheumatic diseases, including systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), systemic sclerosis (SSc), primary Sjogren's syndrome, and idiopathic inflammatory myopathies (IIMs). IIF on Hep-2 cells is considered the gold standard for screening for ANAs. ANA positivity with a titer of ≥ 80 is one of the diagnostic criteria for SLE. The interpretation of Hep-2 IIF results is dependent on the titer and pattern. A titer of 80 has a sensitivity of 98% and specificity of 75% for SLE diagnosis. The antibody pattern can sometimes provide useful information about the disease diagnosis. Positive Hep-2 IIF tests should be confirmed with disease-specific autoantibodies tests. ANA can be present in patients with non-rheumatic diseases and in healthy individuals. A nuclear fine dense fine speckled (DFS70) pattern on HEp-2 can sometimes be observed in 1-8% of healthy individuals. A negative test does not rule out diseases associated with certain antibodies such as SSA/Ro60, Ro52, ribosomal P, Jo1 and some IIM-associated autoantibodies.

References:

Bossuyt, X., De Langhe, E., Borghi, M. O., & Meroni, P. L. Understanding and interpreting antinuclear antibody tests in systemic rheumatic diseases. *Nature Reviews Rheumatology*. 2020; 16(12): 715-726

RHEUMATOID ARTHRITIS PANEL

Seronegative Rheumatoid Arthritis Profile

KSL Beutner Test Code:

- #056 Includes Tests: Rheumatoid Factor (RF) IgM & IgA
Antibodies to Cyclic Citrullinated Peptide (CCP)- IgG
Scavenger Receptor A (SR-A)

Methodology:

- Fluor enzyme immunoassay for RF and CCP: Enzyme Linked Immunosorbent assay (ELISA) for SR-A

Reference Ranges:

- CCP
<20 U/ml = Negative
20-39 U/ml = Weak Positive
40-59 U/ml = Moderate Positive
≥60 U/ml = Strong Positive
- RF – IgA / IgM / IgG
≤6 IU/ml = Negative
>6 IU/ml = Positive
- SR-A
Index value < 0.9: Negative
Index value 0.9 – < 1.0: Indeterminate
Index value ≥ 1.0: Positive

Units:

- IU/ml; U/ml; Index Value

CPT Code:

- 86200, 86431 x2, 83520

Schedule / Turnaround Time:

- Assay performed once weekly. Report availability is within one week from the time of specimen receipt.

Specimen Requirements:

- Collect 2-5 ml of blood in a red top Vacutainer or serum separator (SST) tube, allow blood to clot, and centrifuge. Label tube appropriately.
 - Requested Specimen Volume: 2 mL
 - Absolute Minimum Volume: 0.5 mL
 - Cause for rejection: Specimens other than serum or CSF. Grossly hemolyzed, lipemic or icteric samples

Sample Stability:

- Room temperature = 24 hours
2-8°C = 3 days
15°C to -25°C = up to 3 freeze/thaw cycles

Clinical Relevance:

- Rheumatoid factor (RF) and Cyclic Citrullinated Peptide antibodies (ACPA) are serological biomarkers for diagnosis of Rheumatoid Arthritis (RA). Rheumatoid Factor (RF) is an autoantibody against the Fc portion of immunoglobulin (Ig). Rheumatoid factor IgM, the main isotype identified by RF assays, is found in 70–80% of patients with confirmed RA. The presence of all three RF isotypes (IgG, IgA, and IgM) at abnormal levels has high specificity for a diagnosis of RA. However, presence of RF isotypes in any combination may be found in other rheumatic conditions and in healthy individuals. The three RF isotypes (IgM, IgA, and IgG) are detected in 52% of RA patients but in fewer than 5% patients with other connective tissue diseases.

Anti-CCP positivity, particularly at elevated levels, at any time is associated with higher risk of more severe clinical outcomes, higher disease activity and worse radiographic progression. Anti-CCP can also be present in other disease states. The second generation CCP (CCP2) test has high sensitivity and specificity and is currently recognized as the gold standard of testing for anti-CCP antibodies (ACPA). The accumulated specificity and sensitivity from 164 studies performed from 2002 to 2010 showed a sensitivity in early RA to be 61.6% and 75.2% in established RA and specificity of 94.0%.

The routine biomarkers, i.e., rheumatoid factor (RF) and anticyclic citrullinated peptide antibody (anti-CCP) used in the current classification criteria only show a modest discriminating power. The sensitivity and specificity are 67% and 94% for anti-CCP, and 69% and 85% for RF, respectively. The addition of scavenger receptor -A (SR-A) adds the diagnostic value in RF and CCP seronegative RA patients.

Hu et al. (2020), have shown in a cohort of 179 anti-CCP-negative RA patients, 276 RF-negative RA patients, and 155 (anti-CCP and RF)-double negative RA patients that the positive rates of SR-A in anti-CCP-negative and RF-negative RA patients were 49.72% (89/179) and 39.13% (108/276), respectively. More importantly, in (anti-CCP and RF)-double negative RA patients, SR-A also demonstrated a 42.58% (66/155) prevalence. These results support the use of SR-A to facilitate the diagnosis in anti-CCP and/or RF-negative RA patients. The study also showed that in undifferentiated arthritis patients showed higher levels of SR-A and it increased during disease progression. These findings indicate the potential value of SR-A as a predictor of early RA.

Xie et al., 2022, showed that SR-A levels in patients with RA was significantly higher than that in healthy controls. Moreover, the patients with RA correlated positively with both erosion scores while none of the healthy controls showed elevation of SR-A or joint destruction.

Internal lab studies of 80 rheumatoid arthritis patients who fulfilled the 2010 ACR/EULAR diagnostic criteria, 378 autoimmune disease patients, 70 osteoarthritis and 148 infectious disease patients showed that SR-A sensitivity for RA was 56.3%. The specificity was 88.4%. Of the 24 seronegative patients (RA patients that are negative for RF and CCP) 37.5% were found positive for SR-A alone. Taken together, all these results suggest that SR-A when combined with traditional biomarkers RF and CCP will aid in a more robust serological diagnostic panel for RA. The panel will help in for an earlier and more accurate diagnosis particularly seronegative RA.

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Paalanen, K., Rannio, K., Rannio, T., Asikainen, J., Hannonen, P., & Sokka, T. (2019). Does early seronegative arthritis develop into rheumatoid arthritis? A 10-year observational study. *Clin Exp Rheumatol*, 37(1), 37-43.

Sokolova, M. V., Schett, G., & Steffen, U. (2021). Autoantibodies in rheumatoid arthritis: historical background and novel findings. *Clinical Reviews in Allergy & Immunology*, 1-14.

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